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Buffer- and Diffusion-Mediated Calcium Concentration Fluctuations Accelerate the Stochastic Dynamics of Calcium-Triggered Events

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Ca^{2+} plays a significant role in many cell signaling pathways, some of which are localized to spatially restricted ''domains'' in the cell. Ca^{2+} binding proteins (buffers) play an important role in regulating $[Ca^{2+}]$ and increase the effective volume of these domains. Because fluctuations in $[Ca^{2+}]$ decrease in proportion to the square root of the domain volume, one might expect that buffers decrease $[Ca^{2+}]$ fluctuations and minimize the subsequent influence on Ca^{2+}-dependent signaling. We tested this hypothesis in two settings: (1) closed domains with no influx or efflux of either Ca^{2+} and buffer and (2) open domains with influx and efflux of both Ca^{2+} and buffer. In both settings, we derive the appropriate Langevin system of stochastic differential equations describing the fluctuating dynamics of Ca^{2+} and buffer, determine the size of $[Ca^{2+}]$ fluctuations for different buffer properties, and use Monte Carlo simulation to investigate the influence of buffers on the stochastic dynamics (e.g., the waiting time) for events triggered by rapid sequential binding of Ca^{2+} ions. We find that buffers increase the magnitude of $[Ca^{2+}]$ fluctuations in closed domains, in contrast with a naive application of the concept of effective volume, and buffer-mediated $[Ca^{2+}]$ fluctuations generally decrease the expected waiting time for Ca^{2+}-triggered events. The effect of buffers on $[Ca^{2+}]$ fluctuations in open domains is more complex. In the presence of Ca^{2+} influx, the dynamics of $[Ca^{2+}]$ fluctuations depend on buffer concentration as well as binding and diffusion rates. In general, fast mobile buffers lead to larger fluctuations than slower and less mobile buffers. Our results demonstrate that buffers alter the dynamics of $[Ca^{2+}]$ fluctuations and raise the intriguing question of whether $[Ca^{2+}]$ fluctuations are physiologically significant and perhaps regulated.

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Wnt-11 Signaling in Cardiomyocytes

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The Wnt/frizzled signalling pathways play a key role in cardiogenesis and in adult hearts during cardiac remodelling. We investigated whether the expression of Wnt-11 and its anticipated downstream signalling components is altered in human failing hearts and whether Wnt-11 acutely affects Ca^{2+} transients in adult murine cardiomyocytes.

Left ventricles (LV) homogenates of human non-failing hearts (NF, n=4) and end-stage failing hearts with ischemic (ICM, n=5) and dilated (DCM, n=5) cardiomyopathy were used for western blotting with antibodies against Wnt-11, Fzd-4 and Fzd-7 (Wnt receptors) and Dvl-1 (downstream cytoplasmic regulator). Expression of proteins was normalized to GAPDH and is given as a fraction of average protein expression in NF. Ca^{2+} transients were measured during confocal line scan imaging with Fluo-4 in isolated murine adult cardiomyocytes after 5 (ncells=20) and 25 minutes (ncells=18) incubation with recombinant Wnt-11 (2 µg/ml). Untreated cells served as controls (ncells=27). Wnt-11 expression in LV in ICM (0.85 ± 0.05) and DCM (0.91 ± 0.34) was not significantly changed when compared to NF. However, the expression of Wnt-11 receptors was highly increased: Fzd-4 in ICM (6.25 ± 1.23; $p<0.005$) and in DCM (4.81 ± 0.82; $p<0.005$) as well Fzd-7 in ICM (4.48 ± 0.63; $p<0.001$) and in DCM (4.45 ± 0.76; $p<0.001$). The expression of Dvl-1 tended to be decreased in ICM (0.63 ± 0.15; $p=0.15$) and DCM (0.64 ± 0.29; $p=0.17$). The amplitude of Ca^{2+} transients (F/F0), time to peak, and time to 50 % decay (RT50) were not altered during 5 and 25 minutes incubation with Wnt-11 when compared to untreated control cells.

The expression of the Wnt-receptors, Fzd-4 and Fzd-7 is significantly increased in human end-stage failing hearts, suggesting their regulation in cardiac remodelling and/or dysfunction. Wnt-11 did not acutely alter cardiomyocyte Ca^{2+} transients in non-diseased cardiomyocytes.

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Inhibition of the NA^{+}/CA^{2+} Exchanger (Ncx) Improves Cardiomyocyte Contractile Dysfunction in a Rat Model with Compensated Renal Failure and Heart Failure with Preserved Ejection Fraction

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Background: Heart failure with preserved ejection fraction (HFPEF) and renal impairment are often associated but the underlying pathomechanisms are largely unknown. We investigated tissue fibrosis and cardiomyocyte contractile function in a rat model with HFPEF and compensated renal failure.

Methods: Seventy young male Wistar rats were subjected to subtotal nephrectomy (NXT) or sham operation (SOP). Blood/urine samples, echocardiography, pressure-volume loops were performed after 8 and 24 weeks. Left ventricular (LV) hypertrophy, fibrosis (picosirius-red-staining) and NCX protein expression (Western blot) were determined after sacrification. Cardiomyocyte function (Ca^{2+} transients, cell shortening) was quantified in isolated cardiomyocytes without and with the NCX inhibitor SEA0400 (300nM).

Results: NXT rats showed stable renal impairment with preserved urine excretion and elevated arterial pressure (148 ± 8 and 154 ± 7 vs. 105 ± 3 and 109 ± 2 mmHg, $p<0.01$) at 8 and 24 weeks, respectively. LV in NXT were significantly hypertrophied at 8 and 24 weeks, but LV systolic function (EF, dP/dt) were preserved. LVEDP, LA size and lung weight were significantly increased indicating HFPEF with pulmonary congestion. Fibrosis was increased in LV from NXT. LV cardiomyocytes showed significantly prolonged time for early (50%) relaxation and decay of the Ca^{2+} transient. Time constant of the caffeine-induced Ca^{2+} transient (TAU) was significantly prolonged indicating reduced NCX forward mode activity, while NCX protein expression was upregulated. Acute treatment with SEA0400 (300nM) significantly accelerated early relaxation in cardiomyocytes from NXT (8 weeks).

Conclusion: In this model of compensated renal failure and HFPEF cardiomyocyte relaxation was prolonged already at an early stage. Our results suggest increased NCX activity may contribute to contractile dysfunction, likely through reverse mode Ca^{2+} influx. NCX represents a potential target to treat cardiomyocyte dysfunction in HFPEF.

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Alterations of Nuclear Ca^{2+}-Dependent Signalling in Heart Failure

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A hallmark of heart failure is impaired Ca^{2+} handling of cardiomyocytes. We previously showed that specific alterations in nuclear Ca^{2+} handling precede changes in cytoplasmic Ca^{2+} handling as heart failure progresses. However, a direct link between changes of nucleoplasmic Ca^{2+} handling and altered excitation-transcription coupling during the heart failure progression was not previously established. We thus characterized changes of nuclear Ca^{2+} handling and the activation of nuclear Ca^{2+}-dependent transcription factors under low and high pacing frequencies at the early and late stage of hypertrophy in mouse model of pressure overload.

Ventricular cardiomyocytes were isolated 1 and 7 weeks after transverse aortic constriction (TAC) in adult wild-type mice. Subcellular $[Ca^{2+}]$ transients were recorded in electrically stimulated CMs loaded with Fluo-4/AM. Phosphorylation levels of CaMKII and nuclear accumulation of HDAC4 were quantified by immunocytostaining.

During the early remodelling (i.e. 1 week after TAC intervention) - in contrast to diastolic $[Ca^{2+}]$ in the cytoplasm - diastolic $[Ca^{2+}]$ in the nucleus was already elevated at very low stimulation rate (0.5 Hz) as compared to the non-failing group, and than overproportionally increased with faster stimulation rates. In failing cardiomyocytes (7 weeks after TAC intervention), the changes in nucleoplasmic and cytoplasmic diastolic $[Ca^{2+}]$ were qualitatively comparable, though the increase was more pronounced in the nuclear compartment. High pacing frequency caused significantly higher phosphorylation of CaMKII and corresponding HDAC4 translocation in cardiomyocytes from hypertrophic hearts compared to healthy controls, with the significantly higher increase in CaMKII phosphorylation in the nucleoplasmic compartment as compared to cytoplasm.

In conclusion, we found that the increased stimulation frequency led to a higher build-up of diastolic $[Ca^{2+}]$ in cardiomyocytes from hypertrophied hearts, especially in the nucleoplasmic compartment, which may be involved in the dysregulation of Ca^{2+}-dependent gene transcription and progression of adverse cardiac remodeling.